Response to Office Action dated March 6, 2009

Amendments to the Claims

This Listing of Claims will replace all prior versions and listings of claims in this application.

Listing of Claims

Claims 1-44 (Canceled).

Claim 45 (Currently amended). A culture-independent method of determining the abundance of an environmental parameter in an environmental sample of interest by determining the abundance of at least one nucleic acid marker sequence, wherein the abundance of the nucleic acid marker sequence(s) correlates to the abundance of the environmental parameter, comprising the steps of:

- a. providing a first plurality of environmental samples at least some of which samples contain the environmental parameteran-environmental sample containing a population of interest;
- isolating a <u>plurality of genomic DNAs</u> from the environmental <u>cach of the</u> sample<u>s provided in step a;</u>
- isolating a plurality of 16S rRNA gene segments from each plurality of genomic DNAs isolated in step b;
- d. determining the abundance of each of said 16S rRNA gene segments in each plurality of 16S rRNA gene segments isolated in step c;
- e. determining the abundance of the environmental parameter in each of the samples provided in step.a;
- correlating the abundance of each 16S rRNA gene segment determined in step d with the abundance of the environmental parameter determined in step e;
- g. selecting at least one 16S rRNA gene segment whose abundance correlates to the abundance of said environmental parameter, as determined in step f;

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 h. providing an environmental sample set of at least one environmental sample;

- i. isolating a plurality of genomic DNAs from each environmental sample of the environmental sample set provided in step h;
- [[c.]]i. assaying the genomic DNA by utilizing at least one pair of probes derived from genomic DNA that is specific to at least one of the nucleic acid marker sequences as PCR primers to determine determining the abundance of at least one of the nucleic acid marker sequences in the genomic DNAsaid 16S rRNA gene segment selected isolated from the sample in step g in each plurality of genomic DNAs isolated in step j; and
- [[d,]]k, inferring the abundance of the environmental parameter of interest-in each environmental sample of the environmental sample set provided in step h based upon the abundance of at least one of the nucleic acid marker sequencessaid 16S rRNA gene determined in step j in the genomic DNA isolated from the sample cach environmental sample of the environmental sample set provided in step h.

Claim 46 (Canceled)

Claim 47 (Currently amended). The method according to claim 45-or-elaim 46, wherein the correlation in step g_-the abundance of at least one of the nucleic-acid markers-shows a perfect correlation to the abundance of the parameter of interest, wherein the perfect correlation is expressed by an r-value of 1.

Claim 48 (Currently amended). The method according to claim 45-or-elaim 46, wherein the correlation in step g the abundance of at least one of the nucleic acid markers shows a high degree of correlation to the abundance of the parameter of interest, wherein the high degree of correlation is expressed by an r-value of 0.8 to 0.99.

Claim 49 (Currently amended). The method according to claim 45-or-elaim 46, wherein the correlation in step g the abundance of at least one of the nucleic acid markers shows

a moderate degree of correlation to the abundance of the parameter of interest, wherein the moderate degree of correlation is expressed by an r-value of 0.5 to 0.7.

Claim 50 (Currently amended). The method according to claim 45-or-elaim 46, wherein the environmental parameter of interest is a subsurface oil or natural gas deposit.

Claim 51-57 (Canceled)

Claim 58 (New). The method of claim 45, wherein the determination in step j, of the abundance of said 16S rRNA gene segment, is done using PCR.

Claim 59 (New). The method of claim 45, wherein the determination in step j, of the abundance of said 16S rRNA gene segment, is done using a hybridization assay.

Claim 60 (New). A culture-independent method of identifying an indicator for an environmental parameter, said method comprising the steps of:

- a. providing a plurality of environmental samples at least some of which samples contain the environmental parameter;
- isolating a plurality of genomic DNAs from each of the samples provided in step a;
- isolating a plurality of 16S rRNA gene segments from each plurality of genomic DNAs isolated in step b;
- d. determining the abundance of each of said 16S rRNA gene segments in each plurality of 16S rRNA gene segments isolated in step c;
- determining the abundance of the environmental parameter in each of the samples provided in step a;
- f. correlating the abundance of each 16S rRNA gene segment determined in step d with the abundance of the environmental parameter determined in step e;
- g. selecting at least one 16S rRNA gene segment whose abundance correlates to the abundance of said environmental parameter, as determined in step f;

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h. designating said 16S rRNA gene segment as the indicator.

Claim 61 (New). The method according to claim 60, wherein the correlation in step g is expressed by an r-value of 1.

Claim 62 (New). The method according to claim 60, wherein the correlation in step g is expressed by an r-value of 0.8 to 0.99.

Claim 63 (New). The method according to claim 60, wherein the correlation in step g is expressed by an r-value of 0.5 to 0.7.

 $\label{eq:claim 64 (New). The method according to claim 60, wherein the environmental parameter is subsurface oil or natural gas.$

Claim 65 (New). A culture-independent method of determining the abundance of an environmental parameter in an environmental sample comprising the steps of:

- a. providing at least one environmental sample;
- b. isolating a plurality of genomic DNAs from said environmental sample(s);
- determining the abundance of the indicator identified in the method of claim 60 in the plurality of genomic DNAs isolated in step b; and
- inferring the abundance of the environmental parameter in said sample(s) based upon the abundance of said indicator in said sample(s).

Claim 66 (New). The method according to claim 65, wherein the environmental parameter is subsurface oil or natural gas.

Claim 67 (New). The method of claim 65, wherein the abundance of said indicator in said plurality of genomic DNAs is determined using PCR.

Claim 68 (New). The method of claim 65, wherein the abundance of said indicator in said plurality of genomic DNAs is determined using a hybridization assay.

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Claim 69 (New, Withdrawn). The method of any one of claims 45, 60 or 65, wherein said environmental sample is selected from the group consisting of a body tissue sample, a body fluid sample, a cell culture and a tissue culture, and said environmental parameter is selected from the group consisting of a medical condition, an acute disease state, a chronic disease state, and a physiological state.